

WHAT IS CLAIMED IS:

1           1. An isolated infectious chimeric parainfluenza virus (PIV) comprising  
2 a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large polymerase  
3 protein (L), and a partial or complete PIV vector genome or antigenome combined with one  
4 or more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
5 determinant(s) of one or more heterologous pathogen(s) to form a chimeric PIV genome or  
6 antigenome.

1           2. The chimeric PIV of claim 1, wherein said one or more heterologous  
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are added as  
3 supernumerary gene(s) or genome segment(s) adjacent to or within a noncoding region of  
4 the partial or complete PIV vector genome or antigenome.

1           3. The chimeric PIV of claim 1, wherein said one or more heterologous  
2 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are substituted for  
3 one or more counterpart gene(s) or genome segment(s) in a partial PIV vector genome or  
4 antigenome.

1           4. The chimeric PIV of claim 1, wherein said one or more heterologous  
2 pathogens is a heterologous PIV and said heterologous gene(s) or genome segment(s)  
3 encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or fragment(s)  
4 thereof.

1           5. The chimeric PIV of claim 1, wherein the vector genome or  
2 antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of  
4 one or more heterologous PIV(s).

1           6. The chimeric PIV of claim 5, wherein said one or more heterologous  
2 PIV(s) is/are selected from HPIV1, HPIV2, or HPIV3.

1           7. The chimeric PIV of claim 5, wherein the vector genome or  
2 antigenome is a partial or complete HPIV genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1           8.       The chimeric PIV of claim 7, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1           9.       The chimeric PIV of claim 8, wherein one or more gene(s) or genome  
2 segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from HPIV1  
3 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are added  
4 to or substituted within the partial or complete HPIV3 genome or antigenome.

1           10.      The chimeric PIV of claim 8, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 JS genome or antigenome and the heterologous  
3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of one or more  
4 heterologous HPIV(s).

1           11.      The chimeric PIV of claim 10, wherein one or more gene(s) or  
2 genome segment(s) encoding one or more antigenic determinant(s) of HPIV1 selected from  
3 HPIV1 HN and F glycoproteins and antigenic domains, fragments and epitopes thereof is/are  
4 added to or substituted within the partial or complete HPIV3 JS genome or antigenome.

1           12.      The chimeric PIV of claim 9, wherein both HPIV1 genes encoding  
2 HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes in a partial  
3 HPIV3 vector genome or antigenome.

1           13.      The chimeric PIV of claim 9, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within PIV3 JS *cp45* selected from mutations specifying an amino acid substitution  
4 in the L protein at a position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in  
5 the N protein at a position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C  
6 protein at a position corresponding to Ile96 of JS *cp45*, a nucleotide substitution a 3' leader  
7 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of  
8 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to  
9 nucleotide 62 of JS *cp45*

1           14. The chimeric PIV of claim 8, wherein one or more gene(s) or genome  
2 segment(s) encoding one or more antigenic determinant(s) of HPIV2 is/are added to or  
3 incorporated within the partial or complete HPIV3 genome or antigenome.

1           15. The chimeric PIV of claim 14, wherein one or more HPIV2 gene(s) or  
2 genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic  
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial  
4 or complete HPIV3 vector genome or antigenome.

1           16. The chimeric PIV of claim 6, wherein a plurality of heterologous  
2 genes or genome segments encoding antigenic determinants of multiple heterologous PIVs  
3 are added to or incorporated within the partial or complete HPIV vector genome or  
4 antigenome.

1           17. The chimeric PIV of claim 16, wherein said plurality of heterologous  
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 are  
3 added to or incorporated within a partial or complete HPIV3 vector genome or antigenome.

1           18. The chimeric PIV of claim 17, wherein one or more HPIV1 gene(s) or  
2 genome segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic  
3 domain(s), fragment(s) or epitope(s) thereof and one or more HPIV2 gene(s) or genome  
4 segment(s) encoding one or more HN and/or F glycoprotein(s) or antigenic domain(s),  
5 fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial or  
6 complete HPIV3 vector genome or antigenome.

1           19. The chimeric PIV of claim 18, wherein both HPIV1 genes encoding  
2 HN and F glycoproteins are substituted for counterpart HPIV3 HN and F genes to form a  
3 chimeric HPIV3-1 vector genome or antigenome which is further modified by addition or  
4 incorporation of one or more gene(s) or gene segment(s) encoding one or more antigenic  
5 determinant(s) of HPIV2.

1           20. The chimeric PIV of claim 19, wherein a transcription unit comprising  
2 an open reading frame (ORF) of an HPIV2 HN gene is added to or incorporated within the  
3 chimeric HPIV3-1 vector genome or antigenome.

1                   21. The chimeric PIV of claim 20 selected from rPIV3-1.2HN, or rPIV3-  
2                   1cp45.2HN.

1                   22. The chimeric PIV of claim 1, wherein the vector genome or  
2                   antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3                   heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
4                   respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2  
5                   human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,  
6                   Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
7                   viruses.

1                   23. The chimeric PIV of claim 22, wherein said one or more heterologous  
2                   antigenic determinant(s) is/are selected from measles virus HA and F proteins, subgroup A  
3                   or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps virus HN and F  
4                   proteins, human papilloma virus L1 protein, type 1 or type 2 human immunodeficiency virus  
5                   gp160 protein, herpes simplex virus and cytomegalovirus gB, gC, gD, gE, gG, gH, gI, gJ,  
6                   gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus gp350 protein; filovirus  
7                   G protein, bunyavirus G protein, Flavivirus pre M, E, and NS1 proteins, and alphavirus E  
8                   protein, and antigenic domains, fragments and epitopes thereof.

1                   24. The chimeric PIV of claim 22, wherein the vector genome or  
2                   antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV  
3                   genome or antigenome comprising a partial or complete HPIV3 genome or antigenome  
4                   having one or more gene(s) or genome segment(s) encoding one or more antigenic  
5                   determinant(s) of a heterologous HPIV added or incorporated therein.

1                   25. The chimeric PIV of claim 24, wherein the heterologous pathogen is  
2                   measles virus and the heterologous antigenic determinant(s) is/are selected from the measles  
3                   virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1                   26. The chimeric PIV of claim 25, wherein a transcription unit comprising  
2                   an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within  
3                   a HPIV3 vector genome or antigenome.

1                   27. The chimeric PIV of claim 26 selected from rPIV3(HA HN-L),  
2                   rPIV3(HA N-P), rcp45L(HA N-P), rPIV3(HA P-M), or rcp45L(HA P-M).

1               28.     The chimeric PIV of claim 24, wherein the vector genome or  
2 antigenome is a chimeric HPIV genome or antigenome comprising a partial or complete  
3 HPIV3 genome or antigenome having one or more gene(s) or genome segment(s) encoding  
4 one or more antigenic determinant(s) of HPIV1 added or incorporated therein.

1               29.     The chimeric PIV of claim 25, wherein the heterologous pathogen is  
2 measles virus and the heterologous antigenic determinant(s) is/are selected from the measles  
3 virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1               30.     The chimeric PIV of claim 29, wherein a transcription unit comprising  
2 an open reading frame (ORF) of a measles virus HA gene is added to or incorporated within  
3 a HPIV3-1 vector genome or antigenome having both the HPIV3 HN and F ORFs  
4 substituted by the HN and F ORFs of HPIV1.

1               31.     The chimeric PIV of claim 30, selected from rPIV3-1 HA<sub>P-M</sub> or  
2 rPIV3-1 HA<sub>P-M</sub> cp45L.

1               32.     The chimeric PIV of claim 1, wherein the partial or complete PIV  
2 vector genome or antigenome is combined with one or more supernumerary heterologous  
3 gene(s) or genome segment(s) to form the chimeric PIV genome or antigenome.

1               33.     The chimeric PIV of claim 32, wherein the vector genome or  
2 antigenome is a partial or complete HPIV3 genome or antigenome and said one or more  
3 supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,  
4 HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene  
5 unit.

1               34.     The chimeric PIV of claim 33, wherein one or both of the HPIV1 HN  
2 and/or HPIV2 HN ORF(s) is/are inserted within the HPIV3 vector genome or antigenome,  
3 respectively.

1               35.     The chimeric PIV of claim 33, wherein the HPIV1 HN, HPIV2 HN,  
2 and measles virus HA ORFs are inserted between the N/P, P/M, and HN/L genes,  
3 respectively.

1           36. The chimeric PIV of claim 33, wherein the HPIV1 HN and HPIV2  
2 HN genes are inserted between the N/P and P/M genes, respectively and a 3918-nt GU insert  
3 is added between the HN and L genes.

1           37. The chimeric PIV of claim 33, which is selected from rHPIV3 1HN<sub>N</sub>-  
2 P, rHPIV3 1HN<sub>P-M</sub>, rHPIV3 2HN<sub>N-P</sub>, rHPIV3 2HN<sub>P-M</sub>, rHPIV3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub>, rHPIV3  
3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub> HA<sub>HN-L</sub>, and rHPIV3 1HN<sub>N-P</sub> 2HN<sub>P-M</sub> 3918GU<sub>HN-L</sub>.

4

1           38. The chimeric PIV of claim 32, which contains protective antigens  
2 from one, two, three or four pathogens.

1           39. The chimeric PIV of claim 32, which contains protective antigens  
2 from one to four pathogens selected from HPIV3, HPIV1, HPIV2, and measles virus.

1           40. The chimeric PIV of claim 32, wherein said one or more  
2 supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign  
3 sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to  
4 the wild-type HPIV3 genome length of 15,462 nt.

1           41. The chimeric PIV of claim 32, wherein the addition of said one or  
2 more supernumerary heterologous gene(s) or genome segment(s) specifies an attenuation  
3 phenotype of the chimeric PIV which exhibits at least a 10-to 100-fold decrease in  
4 replication in the upper and/or lower respiratory tract.

1           42. The chimeric PIV of claim 1, wherein the vector genome or  
2 antigenome is a human-bovine chimeric PIV genome or antigenome.

1           43. The chimeric PIV of claim 42, wherein the human-bovine chimeric  
2 vector genome or antigenome is combined with one or more heterologous gene(s) or genome  
3 segment(s) encoding one or more antigenic determinant(s) of a heterologous pathogen  
4 selected from measles virus, subgroup A and subgroup B respiratory syncytial viruses,  
5 mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency viruses,  
6 herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus, filoviruses,  
7 bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1                  44. The chimeric PIV of claim 42, wherein the vector genome or  
2 antigenome comprises a partial or complete HPIV genome or antigenome combined with  
3 one or more heterologous gene(s) or genome segment(s) from a BPIV.

1                  45. The chimeric PIV of claim 44, wherein a transcription unit comprising  
2 an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector genome or  
3 antigenome for a corresponding N ORF of a HPIV3 vector genome.

1                  46. The chimeric PIV of claim 45, wherein the vector genome or  
2 antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1                  47. The chimeric PIV of claim 48, which is rHPIV3-N<sub>B</sub> HA<sub>P-M</sub>.

1                  48. The chimeric PIV of claim 42, wherein the vector genome or  
2 antigenome comprises a partial or complete BPIV genome or antigenome combined with one  
3 or more heterologous gene(s) or genome segment(s) from a HPIV.

1                  49. The chimeric PIV of claim 48, wherein one or more HPIV gene(s) or  
2 genome segment(s) encoding HN and/or F glycoproteins or one or more immunogenic  
3 domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated within the partial  
4 or complete bovine genome or antigenome to form the vector genome or antigenome.

1                  50. The chimeric PIV of claim 49, wherein both HPIV3 genes encoding  
2 HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes to form  
3 the vector genome or antigenome.

1                  51. The chimeric PIV of claim 50, wherein the vector genome or  
2 antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a  
3 supernumerary gene insert.

1                  52. The chimeric PIV of claim 51, which is selected from rBHPIV3-G1 or  
2 rB/HPIV3-F1.

1                  53. The chimeric PIV of claim 49, wherein one or more HPIV1 HN  
2 and/or F gene(s) or genome segment(s) encoding one or more immunogenic domain(s),  
3 fragment(s) or epitope(s) thereof are incorporated within the partial or complete bovine  
4 genome or antigenome to form the vector genome or antigenome, which is further modified

5 by incorporation of one or more HPIV2 HN and/or F gene(s) or genome segment(s)  
6 encoding one or more immunogenic domain(s), fragment(s) or epitope(s) thereof to form the  
7 chimeric genome or antigenome which expresses protective antigen(s) from both HPIV1 and  
8 HPIV2.

1               54. The chimeric PIV of claim 53, which is selected from rB/HPIV3.1-2F;  
2 rB/HPIV3.1-2HN; or rB/HPIV3.1-2F,2HN.

1               55. The chimeric PIV of claim 1, wherein the vector genome or  
2 antigenome is modified to encode a chimeric glycoprotein incorporating one or more  
3 heterologous antigenic domains, fragments, or epitopes of a heterologous PIV or non-PIV  
4 pathogen to form the chimeric genome or antigenome.

1               56. The chimeric PIV of claim 55, wherein the vector genome or  
2 antigenome is modified to encode a chimeric glycoprotein incorporating one or more  
3 antigenic domains, fragments, or epitopes from a second, antigenically distinct PIV to form  
4 the chimeric genome or antigenome.

1               57. The chimeric PIV of claim 55, wherein the chimeric genome or  
2 antigenome encodes a chimeric glycoprotein having antigenic domains, fragments, or  
3 epitopes from two or more HPIVs.

1               58. The chimeric PIV of claim 55, wherein the heterologous genome  
2 segment encodes a glycoprotein ectodomain which is substituted for a corresponding  
3 glycoprotein ectodomain in the vector genome or antigenome.

1               59. The chimeric PIV of claim 55, wherein one or more heterologous  
2 genome segment(s) of a second, antigenically distinct HPIV encoding said one or more  
3 antigenic domains, fragments, or epitopes is/are substituted within a HPIV vector genome or  
4 antigenome to encode said chimeric glycoprotein.

1               60. The chimeric PIV of claim 55, wherein heterologous genome  
2 segments encoding both a glycoprotein ectodomain and transmembrane region are  
3 substituted for counterpart glycoprotein ecto- and transmembrane domains in the vector  
4 genome or antigenome.

1               61.     The chimeric PIV of claim 55, wherein said chimeric glycoprotein is  
2 selected from HPIV HN or F glycoproteins.

1               62.     The chimeric PIV of claim 56, wherein the PIV vector genome or  
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct  
3 PIV is selected from HPIV1 or HPIV2.

1               63.     The chimeric PIV of claim 62, wherein the HPIV vector genome or  
2 antigenome is a partial HPIV3 genome or antigenome and the second, antigenically distinct  
3 HPIV is HPIV2.

1               64.     The chimeric PIV of claim 63, wherein one or more glycoprotein  
2 ectodomain(s) of HPIV2 is/are substituted for one or more corresponding glycoprotein  
3 ectodomain(s) in the HPIV3 vector genome or antigenome.

1               65.     The chimeric PIV of claim 64, wherein both glycoprotein  
2 ectodomain(s) of HPIV2 HN and F glycoproteins are substituted for corresponding HN and  
3 F glycoprotein ectodomains in the HPIV3 vector genome or antigenome.

1               66.     The chimeric PIV of claim 65, which is rPIV3-2TM.

1               67.     The chimeric PIV of claim 55, which is further modified to  
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3 JS *cp45*.

1               68.     The chimeric PIV of claim 55, which is rPIV3-2TM*cp45*

1               69.     The chimeric PIV of claim 55, wherein PIV2 ectodomain and  
2 transmembrane regions of one or both HN and/or F glycoproteins is/are fused to one or more  
3 corresponding PIV3 cytoplasmic tail region(s).

1               70.     The chimeric PIV of claim 69, wherein ectodomain and  
2 transmembrane regions of both PIV2 HN and F glycoproteins are fused to corresponding  
3 PIV3 HN and F cytoplasmic tail regions.

1               71.     The chimeric PIV of claim 70, which is rPIV3-2CT.

1               72. The chimeric PIV of claim 71, which is further modified to  
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3 JS *cp45*.

1               73. The chimeric PIV of claim 72, which is rPIV3-2CT*cp45*.

1               74. The chimeric PIV of claim 55, which is further modified to  
2 incorporate one or more and up to a full panel of attenuating mutations identified in HPIV3  
3 JS *cp45* selected from mutations specifying an amino acid substitution in the L protein at a  
4 position corresponding to Tyr942, Leu992, or Thr1558 of JS *cp45*; in the N protein at a  
5 position corresponding to residues Val96 or Ser389 of JS *cp45*, in the C protein at a position  
6 corresponding to Ile96 of JS *cp45*, a nucleotide substitution in a 3' leader sequence of the  
7 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS *cp45*, and/or  
8 a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS  
9 *cp45*

1               75. The chimeric PIV of claim 55, wherein a plurality of heterologous  
2 genes or genome segments encoding antigenic determinants of multiple heterologous PIVs  
3 are added to or incorporated within the partial or complete HPIV vector genome or  
4 antigenome.

1               76. The chimeric PIV of claim 75, wherein said plurality of heterologous  
2 genes or genome segments encode antigenic determinants from both HPIV1 and HPIV2 and  
3 are added to or incorporated within a partial or complete HPIV3 vector genome or  
4 antigenome.

1               77. The chimeric PIV of claim 55, wherein the chimeric PIV genome or  
2 antigenome is attenuated by addition or incorporation of one or more gene(s) or genome  
3 segment(s) from a bovine PIV3 (BPIV3).

1               78. The chimeric PIV of claim 55, wherein the chimeric genome or  
2 antigenome is modified by introduction of an attenuating mutation involving an amino acid  
3 substitution of phenylalanine at position 456 of the HPIV3 L protein.

1               79. The chimeric PIV of claim 78, wherein phenylalanine at position 456  
2 of the HPIV3 L protein is substituted by leucine.

1               80.     The chimeric PIV of claim 55, wherein the chimeric genome or  
2 antigenome incorporates one or more heterologous gene(s) or genome segment(s) encoding  
3 one or more antigenic determinants from respiratory syncytial virus (RSV) or measles virus.

1               81.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is modified by addition or substitution of one or more heterologous gene(s) or  
3 genome segment(s) that confer increased genetic stability or that alter attenuation,  
4 reactogenicity *in vivo*, or growth in culture of the chimeric virus.

1               82.     The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is modified by introduction of one or more attenuating mutations identified in a  
3 biologically derived mutant PIV or other mutant nonsegmented negative stranded RNA  
4 virus.

1               83.     The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 present within PIV3 JS *cp45*.

1               84.     The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates at least one and up to a full complement of attenuating mutations  
3 specifying an amino acid substitution in the L protein at a position corresponding to Tyr<sub>942</sub>,  
4 Leu<sub>992</sub>, or Thr<sub>1558</sub> of in JS *cp45*; in the N protein at a position corresponding to residues Val<sub>96</sub>  
5 or Ser<sub>389</sub> of JS *cp45*, in the C protein at a position corresponding to Ile<sub>96</sub> of JS *cp45*, in the F  
6 protein at a position corresponding to residues Ile<sub>420</sub> or Ala<sub>450</sub> of JS *cp45*, in the HN protein  
7 at a position corresponding to residue Val<sub>384</sub> of JS *cp45*, a nucleotide substitution a 3' leader  
8 sequence of the chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of  
9 JS *cp45*, and/or a mutation in an N gene start sequence at a position corresponding to  
10 nucleotide 62 of JS *cp45*.

1               85.     The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates attenuating mutations from different biologically derived mutant  
3 PIVs or other mutant nonsegmented negative stranded RNA virus.

1               86.     The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome incorporates an attenuating mutation at an amino acid position corresponding to

3 an amino acid position of an attenuating mutation identified in a heterologous, mutant  
4 negative stranded RNA virus.

1               87. The chimeric PIV of claim 86, wherein said attenuating mutation  
2 comprises an amino acid substitution of phenylalanine at position 456 of the HPIV3 L  
3 protein.

1               88. The chimeric PIV of claim 87, wherein phenylalanine at position 456  
2 of the HPIV3 L protein is substituted by leucine.

1               89. The chimeric PIV of claim 82, wherein the chimeric genome or  
2 antigenome includes at least one attenuating mutation stabilized by multiple nucleotide  
3 changes in a codon specifying the mutation.

1               90. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome comprises an additional nucleotide modification specifying a phenotypic change  
3 selected from a change in growth characteristics, attenuation, temperature-sensitivity, cold-  
4 adaptation, plaque size, host-range restriction, or a change in immunogenicity.

1               91. The chimeric PIV of claim 90, wherein the additional nucleotide  
2 modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes and/or a 3'  
3 leader, 5' trailer, and/or intergenic region within the vector genome or antigenome or within  
4 the heterologous gene(s) or gene segment(s).

1               92. The chimeric PIV of claim 91, wherein one or more PIV gene(s) is  
2 deleted in whole or in part or expression of the gene(s) is reduced or ablated by a mutation in  
3 an RNA editing site, by a frameshift mutation, by a mutation that alters an amino acid  
4 specified by an initiation codon, or by introduction of one or more stop codons in an open  
5 reading frame (ORF) of the gene.

1               93. The chimeric PIV of claim 92, wherein the additional nucleotide  
2 modification comprises a partial or complete deletion of one or more C, D or V ORF(s) or  
3 one or more nucleotide change(s) that reduces or ablates expression of said one or more C, D  
4 or V ORF(s).

1               94. The chimeric PIV of claim 1, wherein the chimeric genome or  
2 antigenome is further modified to encode a cytokine.

1               95. The chimeric PIV of claim 1, which incorporates a heterologous gene  
2 or genome segment from respiratory syncytial virus (RSV).

1               96. The chimeric PIV of claim 95, wherein the heterologous gene or  
2 genome segment encodes RSV F and/or G glycoprotein(s) or immunogenic domain(s),  
3 fragment(s), or epitope(s) thereof.

1               97. The chimeric PIV of claim 1 which is a virus.

1               98. The chimeric PIV of claim 1 which is a subviral particle.

1               99. A method for stimulating the immune system of an individual to  
2 induce protection against PIV which comprises administering to the individual an  
3 immunologically sufficient amount of the chimeric PIV of claim 1 combined with a  
4 physiologically acceptable carrier.

1               100. The method of claim 99, wherein the chimeric PIV is administered in  
2 a dose of  $10^3$  to  $10^7$  PFU.

1               101. The method of claim 99, wherein the chimeric PIV is administered to  
2 the upper respiratory tract.

1               102. The method of claim 99, wherein the chimeric PIV is administered by  
2 spray, droplet or aerosol.

1               103. The method of claim 99, wherein the vector genome or antigenome is  
2 of human PIV3 (HPIV3) and the chimeric PIV elicits an immune response against HPIV1  
3 and/or HPIV2.

1               104. The method of claim 99, wherein the chimeric PIV elicits a  
2 polyspecific immune response against multiple human PIVs and/or against a human PIV and  
3 a non-PIV pathogen.

1               105. The method of claim 99, wherein the vector genome or antigenome is  
2 a partial or complete human PIV (HPIV) genome or antigenome and the heterologous  
3 pathogen is selected from measles virus, subgroup A and subgroup B respiratory syncytial  
4 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency

5                   viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
6                   filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1                   106.     The method of claim 99, wherein the chimeric PIV elicits a  
2                   polyspecific immune response against a human PIV (HPIV) and measles virus.

1                   107.     The method of claim 106, wherein the chimeric PIV elicits a  
2                   polyspecific immune response against HPIV3 and measles virus.

1                   108.     The method of claim 99, wherein a first, chimeric PIV according to  
2                   claim 1 and a second PIV are administered sequentially or simultaneously to elicit a  
3                   polyspecific immune response.

1                   109.     The method of claim 108, wherein the second PIV is a second,  
2                   chimeric PIV according to claim 1.

1                   110.     The method of claim 108, wherein the first, chimeric PIV and second  
2                   PIV are administered simultaneously in a mixture.

1                   111.     The method of claim 108, wherein the first, chimeric PIV and second  
2                   PIV are antigenically distinct variants of HPIV.

1                   112.     The method of claim 111, wherein the first, chimeric PIV comprises a  
2                   partial or complete HPIV3 genome or antigenome combined with one or more heterologous  
3                   gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of a different  
4                   PIV.

1                   113.     The method of claim 111, wherein the first, chimeric PIV and second  
2                   PIV each incorporate one or more heterologous gene(s) or genome segment(s) encoding one  
3                   or more antigenic determinant(s) of a non-PIV pathogen.

1                   114.     The method of claim 113, wherein the first and second chimeric PIV  
2                   incorporate one or more heterologous gene(s) or genome segment(s) encoding one or more  
3                   antigenic determinant(s) of the same non-PIV pathogen.

1                   115.     A method for sequential immunization to stimulate the immune  
2                   system of an individual to induce protection against multiple pathogens comprising  
3                   administering to a newborn to 4 month old infant an immunologically sufficient amount of a

4 first attenuated chimeric HPIV expressing an antigenic determinant of a non-PIV pathogen  
5 and one or more antigenic determinants of HPIV3 and subsequently administering an  
6 immunologically sufficient amount of a second attenuated chimeric HPIV expressing an  
7 antigenic determinant of a non-PIV pathogen and one or more antigenic determinants of  
8 HPIV1 or HPIV2.

1 116. The method for sequential immunization of claim 115, wherein the  
2 first attenuated chimeric HPIV is an HPIV3 expressing a measles virus antigenic determinant  
3 and wherein the second attenuated chimeric HPIV is a PIV3-1 chimeric virus expressing a  
4 measles virus antigenic determinant and incorporating one or more attenuating mutations of  
5 HPIV3 JS *cp45*.

1 117. The method for sequential immunization of claim 115, wherein  
2 following the first vaccination, the vaccinee elicits a primary antibody response against both  
3 PIV3 and the non-PIV pathogen, but not HPIV1 or HPIV2, and upon secondary  
4 immunization the vaccinee is readily infected with the second attenuated HPIV and develops  
5 both a primary antibody response to HPIV1 or HPIV2 and a high titered secondary antibody  
6 response against the non-PIV pathogen.

1 118. The method for sequential immunization of claim 115, wherein the  
2 first chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV  
3 elicits an immune response against HPIV1 or HPIV2, and wherein both the first and second  
4 chimeric PIVs elicit an immune response against measles or RSV.

1 119. The method for sequential immunization of claim 115, wherein the  
2 non-PIV pathogen is selected from measles virus, subgroup A and subgroup B respiratory  
3 syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and type 2 human  
4 immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein  
5 Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1 120. The method for sequential immunization of claim 115, wherein the  
2 second chimeric PIV comprises a partial or complete HPIV3 vector genome or antigenome  
3 combined with one or more gene(s) or genome segment(s) encoding one or more HPIV1  
4 and/or HPIV2 HN and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s)  
5 thereof.

1               121. The method for sequential immunization of claim 115, wherein the  
2 partial or complete vector genome or antigenome of the first, chimeric PIV incorporates at  
3 least one and up to a full complement of attenuating mutations present within HPIV3 JS  
4 cp45 selected from mutations specifying an amino acid substitution in the L protein at a  
5 position corresponding to Tyr942, Leu992, or Thr1558 of JS cp45; in the N protein at a  
6 position corresponding to residues Val96 or Ser389 of JS cp45, in the C protein at a position  
7 corresponding to Ile96 of JS cp45, a nucleotide substitution a 3' leader sequence of the  
8 chimeric virus at a position corresponding to nucleotide 23, 24, 28, or 45 of JS cp45, and/or  
9 a mutation in an N gene start sequence at a position corresponding to nucleotide 62 of JS  
10 cp45.

1               122. An immunogenic composition to elicit an immune response against  
2 PIV comprising an immunogenically sufficient amount of the chimeric PIV of claim 1 in a  
3 physiologically acceptable carrier.

1               123. The immunogenic composition of claim 122, formulated in a dose of  
2  $10^3$  to  $10^7$  PFU.

1               124. The immunogenic composition of claim 122, formulated for  
2 administration to the upper respiratory tract by spray, droplet or aerosol.

1               125. The immunogenic composition of claim 122, wherein the chimeric  
2 PIV elicits an immune response against one or more virus(es) selected from HPIV1, HPIV2  
3 and HPIV3.

1               126. The immunogenic composition of claim 122, wherein the chimeric  
2 PIV elicits an immune response against HPIV3 and another virus selected from HPIV1 and  
3 HPIV2.

1               127. The immunogenic composition of claim 122, wherein the chimeric  
2 PIV elicits a polyspecific immune response against one or more HPIVs and a heterologous  
3 pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial  
4 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency  
5 viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
6 filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses.

1               128. The immunogenic composition of claim 127, wherein the chimeric  
2 PIV elicits a polyspecific immune response against HPIV3 and measles or respiratory  
3 syncytial virus

1               129. The immunogenic composition of claim 122, further comprising a  
2 second, chimeric PIV according to claim 1.

1               130. The immunogenic composition of claim 129, wherein the first and  
2 second chimeric PIVs are antigenically distinct variants of HPIV and bear the same or  
3 different heterologous antigenic determinant(s).

1               131. The immunogenic composition of claim 129, wherein the first  
2 chimeric PIV comprises a partial or complete HPIV3 genome or antigenome combined with  
3 one or more heterologous gene(s) or genome segment(s) encoding one or more antigenic  
4 determinant(s) of a non-PIV heterologous pathogen.

1               132. The immunogenic composition of claim 129, wherein the second  
2 chimeric PIV incorporates one or more heterologous gene(s) or genome segment(s) encoding  
3 one or more antigenic determinant(s) of the same non-PIV heterologous pathogen.

1               133. The immunogenic composition of claim 129, wherein the first  
2 chimeric PIV elicits an immune response against HPIV3 and the second chimeric PIV elicits  
3 an immune response against HPIV1 or HPIV2, and wherein both the first and second  
4 chimeric PIVs elicit an immune response against the non-PIV pathogen.

1               134. The immunogenic composition of claim 129, wherein the  
2 heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
3 respiratory syncytial viruses (RSVs), mumps virus, human papilloma viruses, type 1 and  
4 type 2 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies  
5 virus, Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
6 viruses.

1               135. The immunogenic composition of claim 129, wherein the  
2 heterologous pathogen is selected from measles virus or RSV.

1               136. The immunogenic composition of claim 129, wherein the second  
2 chimeric PIV comprises a partial HPIV3 vector genome or antigenome combined with one

3 or more HPIV1 gene(s) or genome segment(s) encoding one or more antigenic determinants  
4 of HPIV1 HN and/or F glycoproteins.

1               137. The immunogenic composition of claim 129, wherein the second  
2 chimeric PIV compresses a partial or complete HPIV3 vector genome or antigenome  
3 combined with one or more gene(s) or genome segment(s) encoding one or more HPIV2 HN  
4 and/or F glycoprotein(s) or antigenic domain(s), fragment(s) or epitope(s) thereof.

1               138. An isolated polynucleotide comprising a chimeric PIV genome or  
2 antigenome which includes a partial or complete PIV vector genome or antigenome  
3 combined with one or more heterologous gene(s) or genome segment(s) encoding one or  
4 more antigenic determinant(s) of one or more heterologous pathogen(s) to form a chimeric  
5 PIV genome or antigenome.

1               139. The isolated polynucleotide of claim 138, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 added adjacent to or within a noncoding region of the partial or complete PIV vector genome  
4 or antigenome.

1               140. The isolated polynucleotide of claim 138, wherein said one or more  
2 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are  
3 substituted for one or more counterpart gene(s) or genome segment(s) in a partial PIV vector  
4 genome or antigenome.

1               141. The isolated polynucleotide of claim 138, wherein said one or more  
2 heterologous pathogens is a heterologous PIV and said heterologous gene(s) or genome  
3 segment(s) encode(s) one or more PIV N, P, C, D, V, M, F, HN and/or L protein(s) or  
4 immunogenic fragment(s), domain(s), or epitope(s) thereof.

1               142. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3 heterologous gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of  
4 one or more heterologous PIV(s).

1               143 The isolated polynucleotide of claim 142, wherein the vector genome  
2 or antigenome is a partial or complete HPIV3 genome or antigenome and the heterologous

3 gene(s) or genome segment(s) encoding the antigenic determinant(s) is/are of HPIV 1 and/or  
4 HPIV2.

1 144. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome is a partial or complete human PIV (HPIV) genome or antigenome and the  
3 heterologous pathogen is selected from measles virus, subgroup A and subgroup B  
4 respiratory syncytial viruses, mumps virus, human papilloma viruses, type 1 and type 2  
5 human immunodeficiency viruses, herpes simplex viruses, cytomegalovirus, rabies virus,  
6 Epstein Barr virus, filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza  
7 viruses.

1 145. The isolated polynucleotide of claim 144, wherein said one or more  
2 heterologous antigenic determinant(s) is/are selected from measles virus HA and F proteins,  
3 subgroup A or subgroup B respiratory syncytial virus F, G, SH and M2 proteins, mumps  
4 virus HN and F proteins, human papilloma virus L1 protein, type 1 or type 2 human  
5 immunodeficiency virus gp160 protein, herpes simplex virus and cytomegalovirus gB, gC,  
6 gD, E, gG, gH, gI, gJ, gK, gL, and gM proteins, rabies virus G protein, Epstein Barr Virus  
7 gp350 protein; filovirus G protein, bunyavirus G protein, Flavivirus E and NS1 proteins, and  
8 alphavirus E protein, and antigenic domains, fragments and epitopes thereof.

1 146. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome is a partial or complete HPIV3 genome or antigenome or a chimeric HPIV  
3 genome or antigenome comprising a partial or complete HPIV3 genome or antigenome  
4 having one or more gene(s) or genome segment(s) encoding one or more antigenic  
5 determinant(s) of a heterologous HPIV added or incorporated therein.

1 147. The isolated polynucleotide of claim 146, wherein the heterologous  
2 pathogen is measles virus and the heterologous antigenic determinant(s) is/are selected from  
3 the measles virus HA and F proteins and antigenic domains, fragments and epitopes thereof.

1 148. The isolated polynucleotide of claim 147, wherein a transcription unit  
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or  
3 incorporated within a HPIV3 vector genome or antigenome.

1 149. The isolated polynucleotide of claim 147, wherein a transcription unit  
2 comprising an open reading frame (ORF) of a measles virus HA gene is added to or

3 incorporated within a HPIV3-1 vector genome or antigenome having both the HPIV3 HN  
4 and F ORFs substituted by the HN and F ORFs of HPIV1.

1               150. The isolated polynucleotide of claim 138, wherein the partial or  
2 complete PIV vector genome or antigenome is combined with one or more supernumerary  
3 heterologous gene(s) or genome segment(s) to form the chimeric PIV genome or  
4 antigenome.

1               151. The isolated polynucleotide of claim 150, wherein the vector genome  
2 or antigenome is a partial or complete HPIV3 genome or antigenome and said one or more  
3 supernumerary heterologous gene(s) or genome segment(s) are selected from HPIV1 HN,  
4 HPIV1 F, HPIV2 HN, HPIV2 F, measles HA, and/or a translationally silent synthetic gene  
5 unit.

1               152. The isolated polynucleotide of claim 138, wherein one, two or all of  
2 the HPIV1 HN, HPIV2 HN, and measles virus HA ORFs are added to the vector genome or  
3 antigenome.

1               153. The isolated polynucleotide of claim 138, wherein one or more of the  
2 HPIV1 HN and HPIV2 HN genes and a 3918-nt GU insert is/are added are added to the  
3 vector genome or antigenome.

1               154. The isolated polynucleotide of claim 150, wherein said one or more  
2 supernumerary heterologous gene(s) or genome segment(s) add a total length of foreign  
3 sequence to the recombinant genome or antigenome of 30% to 50% or greater compared to  
4 the wild-type HPIV3 genome length of 15,462 nt.

1               155. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome is a human-bovine chimeric PIV genome or antigenome.

1               156. The isolated polynucleotide of claim 155, wherein the human-bovine  
2 chimeric vector genome or antigenome is combined with one or more heterologous gene(s)  
3 or genome segment(s) encoding one or more antigenic determinant(s) of a heterologous  
4 pathogen selected from measles virus, subgroup A and subgroup B respiratory syncytial  
5 viruses, mumps virus, human papilloma viruses, type 1 and type 2 human immunodeficiency  
6 viruses, herpes simplex viruses, cytomegalovirus, rabies virus, Epstein Barr virus,  
7 filoviruses, bunyaviruses, flaviviruses, alphaviruses and influenza viruses

1               157. The isolated polynucleotide of claim 156, wherein the vector genome  
2 or antigenome comprises a partial or complete HPIV genome or antigenome combined with  
3 one or more heterologous gene(s) or genome segment(s) from a BPIV.

1               158. The isolated polynucleotide of claim 157, wherein a transcription unit  
2 comprising an open reading frame (ORF) of a BPIV3 N ORF is substituted in the vector  
3 genome or antigenome for a corresponding N ORF of a HPIV3 vector genome.

1               159. The isolated polynucleotide of claim 158, wherein the vector genome  
2 or antigenome is combined with a measles virus HA gene as a supernumerary gene insert.

1               160. The isolated polynucleotide of claim 138, wherein the vector genome  
2 or antigenome comprises a partial or complete BPIV genome or antigenome combined with  
3 one or more heterologous gene(s) or genome segment(s) from a HPIV.

1               161. The isolated polynucleotide of claim 160, wherein one or more HPIV  
2 gene(s) or genome segment(s) encoding HN and/or F glycoproteins or one or more  
3 immunogenic domain(s), fragment(s) or epitope(s) thereof is/are added to or incorporated  
4 within the partial or complete bovine genome or antigenome to form the vector genome or  
5 antigenome.

1               162. The isolated polynucleotide of claim 161, wherein both HPIV3 genes  
2 encoding HN and F glycoproteins are substituted for corresponding BPIV3 HN and F genes  
3 to form the vector genome or antigenome.

1               163. The isolated polynucleotide of claim 162, wherein the vector genome  
2 or antigenome is combined with a respiratory syncytial virus (RSV) F or G gene as a  
3 supernumerary gene insert.

1               164. The isolated polynucleotide of claim 138, wherein the chimeric  
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,  
3 fragments, or epitopes from both a human PIV (HPIV) and a heterologous pathogen.

1               165. The isolated polynucleotide of claim 164, wherein the chimeric  
2 genome or antigenome encodes a chimeric glycoprotein having antigenic domains,  
3 fragments, or epitopes from two or more different PIVs.

1               166. The isolated polynucleotide of claim 138, wherein the chimeric  
2 genome or antigenome is modified by introduction of one or more attenuating mutations  
3 identified in a biologically derived mutant PIV or other mutant nonsegmented negative  
4 stranded RNA virus.

1               167. The isolated polynucleotide of claim 138, wherein, the chimeric  
2 genome or antigenome incorporates at least one and up to a full complement of attenuating  
3 mutations present within PIV3 JS *cp45*.

1               168. The isolated polynucleotide of claim 138, wherein the chimeric  
2 genome or antigenome incorporates an attenuating mutation from a heterologous  
3 nonsegmented negative stranded RNA virus.

1               169. The isolated polynucleotide of claim 138, wherein the chimeric  
2 genome or antigenome comprises an additional nucleotide modification specifying a  
3 phenotypic change selected from a change in growth characteristics, attenuation,  
4 temperature-sensitivity, cold-adaptation, plaque size, host-range restriction, or a change in  
5 immunogenicity.

1               170. The isolated polynucleotide of claim 138, wherein the additional  
2 nucleotide modification alters one or more PIV N, P, C, D, V, M, F, HN and/or L genes  
3 and/or a 3' leader, 5' trailer, and/or intergenic region within the vector genome or  
4 antigenome or within the heterologous gene(s) or gene segment(s).

1               171. The isolated polynucleotide of claim 138, wherein one or more PIV  
2 gene(s) is deleted in whole or in part or expression of the gene(s) is reduced or ablated by a  
3 mutation in an RNA editing site, by a frameshift mutation, by a mutation that alters an amino  
4 acid specified by an initiation codon, or by introduction of one or more stop codons in an  
5 open reading frame (ORF) of the gene.

1               172. A method for producing an infectious attenuated chimeric PIV particle  
2 from one or more isolated polynucleotide molecules encoding said PIV, comprising:

3               expressing in a cell or cell-free lysate an expression vector comprising an  
4 isolated polynucleotide comprising a partial or complete PIV vector genome or antigenome  
5 of a human or bovine PIV combined with one or more heterologous gene(s) or genome

6 segment(s) encoding one or more antigenic determinant(s) of one or more heterologous  
7 pathogen(s) to form a chimeric PIV genome or antigenome, and PIV N, P, and L proteins.

1               173. The method of claim 172, wherein the chimeric PIV genome or  
2 antigenome and the N, P, and L proteins are expressed by two or more different expression  
3 vectors.

1               174. An expression vector comprising an operably linked transcriptional  
2 promoter, a polynucleotide sequence which includes a partial or complete PIV vector  
3 genome or antigenome of a human or bovine PIV combined with one or more heterologous  
4 gene(s) or genome segment(s) encoding one or more antigenic determinant(s) of one or more  
5 heterologous pathogen(s) to form a chimeric PIV genome or antigenome, and a  
6 transcriptional terminator.

1               175. An isolated infectious recombinant parainfluenza virus (PIV)  
2 comprising a major nucleocapsid (N) protein, a nucleocapsid phosphoprotein (P), a large  
3 polymerase protein (L), and a PIV genome or antigenome having a polynucleotide insertion  
4 of between 150 nucleotides (nts) and 4,000 nucleotides in length in a non-coding region  
5 (NCR) of the genome or antigenome or as a separate gene unit (GU), said polynucleotide  
6 insertion lacking a complete open reading frame (ORF) and specifying an attenuated  
7 phenotype in said recombinant PIV.

1               176. The recombinant PIV of claim 175, wherein said polynucleotide insert  
2 is introduced into the PIV genome or antigenome in a reverse, non-sense orientation  
3 whereby the insert does not encode protein.

1               177. The recombinant PIV of claim 175, wherein said polynucleotide insert  
2 is approximately 2,000 nts or greater in length.

1               178. The recombinant PIV of claim 175, wherein said polynucleotide insert  
2 is approximately 3,000 nts or greater in length.

1               179. The recombinant PIV of claim 175, wherein said recombinant PIV  
2 replicates efficiently *in vitro* and exhibits an attenuated phenotype *in vivo*.